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(54) Title: LIPID PRODRUGS OF SALICYLATE AND NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

(57) Abstract

The present invention provides phospholipid prodrugs of aspirin, other salicylates, and nonsteroidal anti-inflammatory drugs useful in the therapy of chronic inflammatory disorders. The drugs are linked to either or both of the glycerol hydroxyls of a phospholipid or to available hydroxyls of phospholipid head groups by ester bonds. They may also be linked to available amines of phospholipid head groups by amine bonds. Drugs linked to phospholipid glycerol are released in vivo by the action of phospholipase A_2 and other phospholipases, while those linked to the phospholipid head groups are released by other endogenous hydrolases. The phospholipid prodrugs reduce the gastrointestinal irritation and toxicity of these drugs when administered at high doses. The prodrugs further provide sustained serum levels of the drugs and allow longer intervals between doses through metabolically controlled release of the active agent.

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LIPID PRODRUGS OF SALICYLATE AND NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Background of the Invention

The present invention relates to lipid derivatives of anti-inflammatory drugs that reduce the toxicity of these agents in the gastrointestinal tract when they are used at high doses and for extended periods. It specifically relates to phospholipid prodrugs of aspirin, other salicylates, and the group of drugs classified as nonsteroidal anti-inflammatory drugs (NSAIDs).

Patients who suffer from chronic inflammatory disease such as rheumatoid arthritis and osteoarthritis must take anti-inflammatory drugs to obtain relief from chronic pain and also to reduce the inflammatory response which can damage joints. Anti-inflammatory drugs in common use are aspirin and other derivatives of salicylic acid as well as nonsteroidal anti-inflammatory agents comprising, for example, Motrin™ (Upjohn, Kalamazoo, Michigan 49001), Naprosyn™ (Syntex, Palo Alto, California 94303), Voltaren™ (Ciba-Geigy, Summit, New Jersey 07901) and Clinoril™ (Merck Sharpe and Dohme, West Point, Pennsylvania 19486). Chronic ingestion of these drugs at high doses is irritating to the gastrointestinal tract. Many patients on this therapy develop single or multiple ulcerations involving the esophagus, stomach, duodenum, and the small and large intestines. Ulceration can proceed to perforation and hemorrhage in these areas, often with life-threatening and even fatal consequences. Even patients who do not experience ulceration frequently complain of nausea associated with the irritating effects of the drugs.

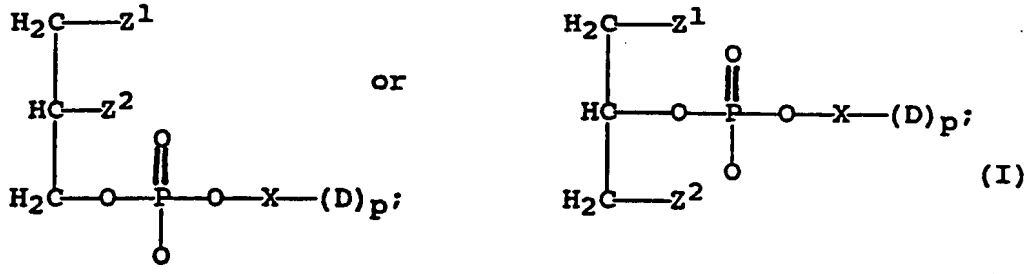
It is advantageous therefore to administer these agents in forms which are less irritating to the stomach and other regions of the gastrointestinal tract. Triglyceride

derivatives of aspirin have been synthesized, and these are reported to produce less gastric ulceration at comparable dosages of drug (Kumar, R. and J.D. Billmoria, J. Pharm. Pharmac. 30:754-758 (1978); U.S. Patent No. 3,686,238 to A. Zaffaroni; U.S. Patent No. 3,644,424 to M. Sherlock; Offenlegungschrift 2,549,783 to D. Germaise). One mechanism by which the reduced toxicity occurs could be the delayed release of active drug which depends on the action of endogenous lipase in the digestive tract.

It would be desirable to develop other derivatives of the above described anti-inflammatory agents whose toxicity in the gastrointestinal tract remains acceptable at doses that are high enough to maintain efficacy, and from which release of the free drug would occur at a slow rate under the influences of endogenous metabolic processes.

Summary of the Invention

This invention is directed to novel compounds of the formula I:



comprising an anti-inflammatory drug linked to a phospholipid and pharmaceutically suitable salts thereof wherein the various substituents are as defined herein below.

This invention provides compounds to reduce the toxicity or irritating effect of aspirin, other salicylates, and non-steroidal anti-inflammatory agents on the gastrointestinal tract. The compounds of the invention can

be administered in conventional pharmaceutical preparations for enteral use or in the form of a liposomal preparation.

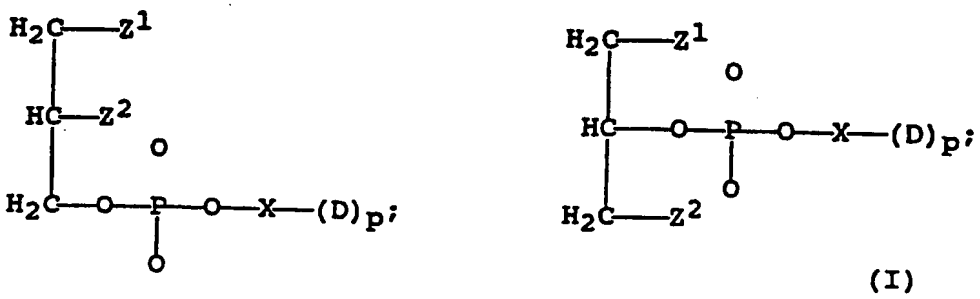
The invention provides a method of synthesizing a phospholipid derivative of an anti-inflammatory drug, comprising the step of reacting one of these drugs, having a functional linking group, with a phospholipid in the presence of a coupling reagent, whereby the drug is linked to the phospholipid to form a lipid prodrug having the structure of Formula I. The anti-inflammatory drug has a free carboxylic group and is linked to the glycerol moiety of the phospholipid through an ester bond or to the phospholipid head group through an ester or amide bond. Accordingly, the phospholipid to which the drug is linked can be a diacylphospholipid, a 1-acyl, 2-lysophospholipid, a 1-lyso, 2-acylphospholipid, or glycerol phosphate.

The invention also provides a method for treating a patient having a chronic inflammatory disease, comprising the administration of an effective, inflammation-reducing amount of a phospholipid prodrug having the structure of Formula I, and repeating the administration at regular intervals and for a period of time sufficient to alleviate the inflammatory symptoms of the disease.

Detailed Description of the Invention

We have developed phospholipid derivatives of salicylates and nonsteroidal anti-inflammatory drugs (NSAIDs) which will greatly reduce the toxicity of these agents in the gastrointestinal tract. These phospholipid prodrugs comprise embodiments having an anti-inflammatory drug molecule, in either monomer or dimer form, attached to either the glycerol moiety of the phospholipids, the phospholipid polar head group, or attached at both sites. The embodiments having a drug molecule attached to the phospholipid glycerol moiety will be acted upon by digestive enzymes such as phospholipase A_2 and other

phospholipases and lysophospholipases, and those having the drug attached at the phospholipid head group will be acted upon by various hydrolases in the body to release the free drug slowly, thus providing a steady level of the drug in the bloodstream while reducing toxicity. The embodiments of phospholipid prodrugs having a salicylate or nonsteroidal anti-inflammatory drug attached to a phospholipid have the general formula:



(I)

wherein D is a salicylate or a nonsteroidal anti-inflammatory drug;

Z^1 and Z^2 are independently $(D)_n$, OH, or R,

wherein R is a C_2 to C_{24} aliphatic group in ester, ether, or vinyl ether linkage, having from 0 to 6 sites of unsaturation and the structure:

$CH_3-(CH_2)_a-(CH=CH-CH_2)_b-(CH_2)_c-Y$

and wherein the sum of a and c is from 1 to 23; and b is 0 to 6; and wherein Y is C(O)O-, C-O-, C=C-O-, C(O)S, C-S, or C=C-S;

except Z^1 and Z^2 are not both OH;

n = 0 to 2; p = 0 to 2; p + n > 0; and

X is a phospholipid head group or is absent, providing that when X is absent, p = 0.

Anti-inflammatory drugs to be incorporated into phospholipid prodrugs according to the invention may be any anti-inflammatory agent having an unesterified carboxylic group. These agents may therefore comprise (1) propionic acid derivatives; (2) acetic acid derivatives; (3) fenamic

acid derivatives; (4) biphenylcarboxylic acid derivatives; or (5) salicylates; and in preferred embodiments of the invention comprise 1-acetylsalicylic acid (aspirin; Bayer); (Z)-5-fluoro-2-methyl-1-[[p-(methylsulfinyl)phenyl]methylene]1-H-indene-3-acetic acid (sulindac), available as Clinoril™ (Merck, Sharpe and Dohme, West Point, Pennsylvania 19486); 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid, monosodium salt (diclofenac), available as Voltaren™ (Ciba-Geigy, Summit, New Jersey); 2',4'-difluoro-4-hydroxy-3-biphenylcarboxylic acid (diflunisal), available as Dolobid™, (Merck, Sharpe and Dohme); 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid (indomethacin), available as Indocin™ (Merck, Sharpe and Dohme); (±)-2-(p-isobutylphenyl)propionic acid (ibuprofen), available as Advil™ (Whitehall Laboratories, Inc., New York, NY 10017); N-(2), 6-dichloro-m-tolyl) anthranilic acid (meclophenomate), available as Meclomen™ (Parke-Davis, Morris Plains, New Jersey 07950; fenoprofen, an arylacetic acid derivative, available as Nalfon™ (Dista Products Co., Indianapolis, Indiana 46285; 2-naphthaleneacetic acid, 6-methoxy-alpha-methyl-,(+) (naproxyn), available as Naprosyn™ (Syntex, Palo Alto, California 94303); 1-methyl-5-(4-methylbenzoyl)-1H-pyrrole-2-acetate dihydrate (tolmetin), available as Tolectin™ (McNeil Pharmaceutical, Spring House, Pennsylvania 19477); and derivatives and congeners thereof. An appendix lists nonsteroidal anti-inflammatory agents presently on the market and their manufacturers. The available carboxyl group by which these drugs may be attached to phospholipids according to the present invention is indicated where structural drawings appear.

The phospholipid moiety of the prodrug may be any physiologically acceptable species that is biochemically available to the action of endogenous phospholipases.

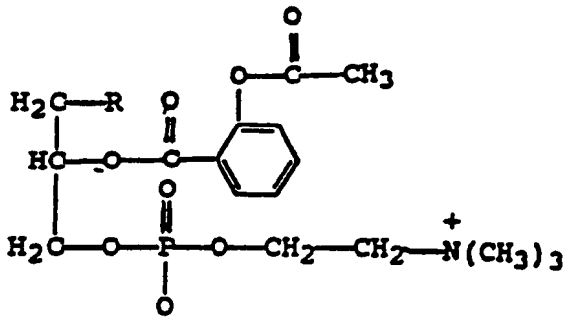
Accordingly, the phospholipid to which the drug is linked can be a diacylphospholipid, a 1-acyl, 2-lysophospholipid, a 1-lyso, 2-acylphospholipid, or a glycerol phosphate. The phospholipid can be a phosphatidyl ester, having a headgroup comprising such species as choline, ethanolamine or serine, or it can be a phosphatidic acid. In preferred embodiments, the phospholipid is any of the physiological phospholipids; for example, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, or phosphatidylserine. The phospholipid may also be a synthetic phosphoglyceride. The stereochemistry of the glycerol phosphate moieties can include sn-1 or sn-3 glycerol phosphate bonds or racemic mixtures thereof; however, sn-glycerol-3-phosphate species are preferred because digestive phospholipase is specific for, and therefore acts preferentially on, that species. Derivatives of sn-glycerol-2-phosphate are also possible.

In preferred embodiments, the anti-inflammatory drug, D, is linked to the phospholipid by an ester bond between a carboxylic acid group of the drug and an hydroxyl of the phospholipid glycerol moiety.

The phospholipid moeity of the prodrug may also comprise a C_2 to C_{24} aliphatic group, R, which may be saturated or unsaturated. It can be attached to the glycerol moiety by an ester, ether or vinyl ether linkage. In preferred embodiments, the R group is a fatty acid and in most preferred embodiments, these aliphatic groups in acyl ester linkage comprise naturally occurring fatty acids, such as lauric, myristic, palmitic, stearic, arachidic and lignoceric, and the naturally occurring unsaturated fatty acids palmitoleic, oleic, linoleic, linolenic and arachidonic. In other embodiments, the aliphatic group can be a branched chain of the same carbon number, and comprise

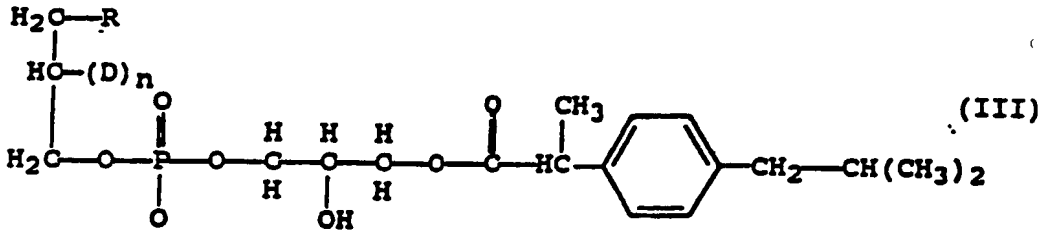
primary or secondary alkanol or alkoxy groups, cyclopropane groups, and internal ether linkages.

In a particularly preferred embodiment of the invention, aspirin is attached by an ester linkage to the sn-2 hydroxyl of phosphatidylcholine, and has the formula:



(II)

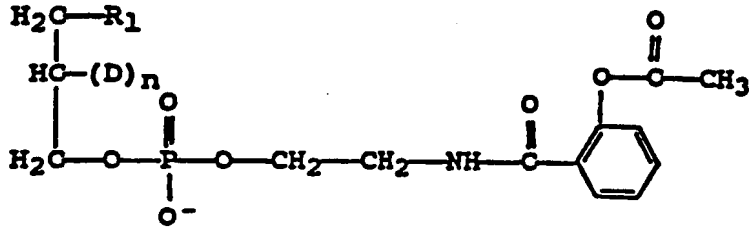
Structures of other phospholipid prodrugs comprise those in which a salicylate or a non-steroidal anti-inflammatory agent is attached to the phospholipid through the phospholipid head group. The drug can be attached, according to one embodiment, by an ester bond joining a carboxyl group of the drug to an available hydroxyl of the head group; for example, one of the glycerol hydroxyl groups of phosphatidylglycerol, the serine hydroxyl of phosphatidylserine or one or more of the inositol hydroxyls of phosphatidylinositol. A prodrug of the invention comprising ibuprofen attached at one of the hydroxyl groups of phosphatidylglycerol has the formula:



(III)

wherein R is as defined above.

Alternatively, the drug can be attached to the phospholipid by an amide bond joining a carboxyl group of the drug to an available amine of the polar head group; for example, the amine of phosphatidylserine or phosphatidylethanolamine. A prodrug of the invention having aspirin attached to the amine group of phosphatidylethanolamine has the formula:



(IV)

wherein R is as defined above.

In other embodiments of the invention, anti-inflammatory drug molecules are attached at both the glycerol moiety and the head group of the phospholipid. Where two or more drug molecules are present on the same phospholipid structure, these drug molecules may be the same or different. Lipid prodrugs comprising dimers of drugs may also be prepared. Dimers of the drug can be linked to either or both hydroxyl groups of the glycerol moiety or to an hydroxyl or amine group of a phospholipid head group in the same way as the monomers are linked.

Synthesis of Phospholipid Prodrugs

Compositions of one embodiment of the invention are made by joining a carboxyl functional group of a salicylate or a nonsteroidal anti-inflammatory drug to an available hydroxyl of a phospholipid through an ester linkage or to an available amine group through an amide linkage. In a preferred embodiment, the available hydroxyl group to which the drug is linked is at the 1, 2, or 3 positions of the glycerol moiety, for example, at the 1-position of a 1-

lyso, 2-acylphospholipid or a 1-lyso, 3-acylphospholipid; alternatively the drug can be linked through an ester bond to an available hydroxyl of the phospholipid head group.

The esterification synthesis is carried out according to two general procedures, one involving the use of an acyl chloride of the drug, and in another approach, one involving the preliminary formation of an acid anhydride.

The acyl chloride procedure is essentially that of Kumar, R. and J. Billimoria, J. Pharm. Pharmac. 30:754-758 (1978) for preparing glycerol acetylsalicylate. The method for preparing phospholipid analogues is described in Example 1 for the synthesis of the 2-aspirin derivative of phosphatidylcholine. The carboxyl group of the drug is first converted to an acyl chloride by means of oxalyl chloride or the drug is obtained in chloride form from a supplier. The esterification then is carried out in a one-step, single-phase reaction in a mixture of chloroform and pyridine. The reaction is stopped and the product is crystallized from the chloroform phase and further purified. The reactive chloride form of the drug can also esterify available hydroxyl groups on other drug molecules to form phospholipid-linked drug dimers, as noted in Example 1.

According to an alternative synthetic approach illustrated in Examples 2 and 3, a phospholipid hydroxyl can be esterified using a symmetrical acid anhydride of the drug. The anhydride can be prepared in a preliminary reaction by the dicyclohexycarbodiimide (DCC) condensation method of Selinger, Z. and Lapidot, Y. J. Lipid Research 7:175-176 (1966). The prepared anhydride is then added to a lysophosphatide or glycerol phosphate in the presence of pyridine and 4-dimethylaminopyridine in chloroform and allowed to react, preferably under nitrogen. The product is isolated either by precipitation in ether or evaporation of the washed chloroform phase, redissolved in chloroform

and purified by chromatography. The anhydride method was applied in the preparation of a salicyloyl phosphatidylcholine (Example 2) and a 2-ibuprofen phosphatidylcholine (Example 3), as well as a 2-acetyl salicyloyl phosphatidylcholine (Example 1). Experimental results in the synthesis of the aspirin derivative indicate that substitution of the anhydride for aspirin chloride and use of 4-dimethylaminopyridine in the esterification reaction substantially increases the yield of the reaction compared to the acyl chloride procedure.

In another embodiment of the invention, anti-inflammatory drugs are attached to the available hydroxyls of phospholipid head groups, using the synthetic procedures described and carrying out the esterification step on 1,2-diacyl phosphatides, or on lyso phosphatides to which drug molecules have been previously attached at the glycerol moiety.

Other embodiments of phospholipid prodrugs can be synthesized by forming an amide link between the available amine group of a phospholipid head group, such as that of ethanolamine, and the carboxylic acid group of a suitable anti-inflammatory drug. The synthesis can be carried out by reacting the carboxyl group of the drug in the form of the acid with the amine group of the phospholipid in the presence of an agent such as dicyclohexylcarbodiimide (DCC); Aldrich, Milwaukee, Wisconsin), or by reacting the acid anhydride of the drug with the same amine group in the presence of an agent such as 4-methylaminopyridine, as described for the synthesis of a diacyl phosphatidyl ethanolamine derivative of aspirin in Example 4.

The phospholipid and anti-inflammatory drug to be joined as a phospholipid prodrug are advantageously reacted, according to any of the procedures described, in lipid:drug molar ratios of from about 1:4 to 4:1, preferably in the ratios of 1:1 or 1:2 as determined by the structure of the

desired product. Excess quantities of either reactant can be used to improve the rate of reaction or to increase yield, as is known to those skilled in the art.

Lipid prodrugs of anti-inflammatory agents having a drug molecule attached both to the glycerol moiety and the head group of a phospholipid can be synthesized by performing simultaneous or successive attachment of the drug molecules through appropriate selection of the above synthetic procedures as described in Example 5. A successive attachment of drug molecules, one to the glycerol moiety by means of an ester linkage and one to the phospholipid head group by means of an amide linkage, can be used to synthesize a lipid prodrug comprising two different drug molecules.

Therapeutic Use of Lipid Prodrugs

When a lipid prodrug having a drug molecule attached at the sn-2 position of a phospholipid is administered to a patient, the drug ester is hydrolyzed by the digestive enzyme, phospholipase A_2, releasing the active compound to be absorbed into the bloodstream. Where the drug is esterified at the sn-1 hydroxyl alone, the groups at the sn-2 position may be hydroxyl or an R group. In these embodiments, when sn-2 is hydroxyl, the drug ester may be hydrolyzed by a lysophospholipase enzyme found in pancreatic secretions to release the free drug *in vivo*. In other embodiments, for example, where the drug is attached to the phospholipid head group, the drug may be released from the phospholipid by other endogenous hydrolases.

The phospholipid prodrugs of the invention are distinct from triglyceride derivatives in their route of absorption. They are also distinct in metabolism because triglyceride-linked drugs are hydrolyzed by lipase enzymes rather than phospholipases or lysophospholipases, and these lipase enzymes act only on esters at the 3-position of glycerol.

The lipid prodrugs of the present invention can be used to enhance the therapy of patients who require anti-inflammatory medication by reducing toxicity of drug doses comparable to conventional therapy, providing a more sustained serum level of medication rather than peaks and troughs of serum concentrations, and by allowing less frequent dosing. By administration of a single prodrug or a combination of prodrugs having drugs esterified at either of the two available phospholipid glycerol positions, or at the phospholipid head group, all of which are subject to hydrolysis by different enzyme systems, the serum concentration of the drug and the dosing intervals can be optimized. Dosage and duration of the drug effect can also be adjusted, by administering, or adding in combination, prodrugs having two or more drug molecules attached to each phospholipid. In embodiments of the invention wherein two drugs are attached to the same phospholipid molecule, or where two or more prodrugs are added in combination, the drugs attached to the phospholipid molecules may be the same or different. In addition, therapeutic formulations may contain some free drug, if required to produce a more rapid increase in serum level.

Lipid prodrugs of anti-inflammatory agents are also useful in enhancing the oral administration of peptides in liposomal form such as disclosed in U.S. Patent No. 4,692,433, entitled "Method and Composition for Regulating the Serum Calcium Levels of Mammals." Further, since the phospholipid prodrugs themselves are amphipathic and capable of forming liposomes they may be incorporated into liposomes comprising other therapeutic agents or combined therewith as a distinct liposomal preparation.

The lipid prodrugs may be administered as such in or in liposomal formulations according to conventional formulations for oral ingestion known to the pharmacy art. Pharmaceutical preparations containing lipid prodrugs may

be prepared by conventional dissolving and lyophilizing processes to contain from approximately 0.1% to 100%, preferably from 0.1% to 50%, of the active ingredient. They can be prepared in the form of tablets, capsules, pills, ampoules of powdered active agent, or oily or aqueous suspensions or solutions. Tablets or other non-liquid oral compositions may contain acceptable excipients, known to the art for the manufacture of pharmaceutical compositions, comprising diluents, such as lactose or calcium carbonate; binding agents such as gelatin or starch; and one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring or preserving agents to provide a palatable preparation. Moreover, such oral preparations may be coated by known techniques to further delay disintegration and absorption in the intestinal tract.

Aqueous suspensions may contain the active ingredient in admixture with pharmacologically acceptable excipients, comprising suspending agents, such as methyl cellulose; and wetting agents, such as lecithin or long-chain fatty alcohols. The said aqueous suspensions may also contain preservatives, coloring agents, flavoring agents and sweetening agents in accordance with industry standards.

The invention can be better understood by way of the following examples which are representative of the preferred embodiments thereof, but which are not to be construed as limiting the scope of the invention.

EXAMPLE 1

Synthesis of 1-acyl,2-(2'-acetoxycarboxybenzoyl)-*sn*-glycero-3-phosphocholine

A quantity of 50 mg of 1-acyl, 2-lysophosphatidylcholine (from egg) (Avanti Polar Lipids, Birmingham, Alabama) was dissolved in 20 ml of dry chloroform and 5 ml of dry

pyridine and 29 mg of aspirin chloride (Fluka Chemical Co., Ronkonkoma, NY), dissolved in 5 ml of dry chloroform, was added slowly over a period of 30 minutes at room temperature with stirring. The mixture was stirred overnight and the reaction stopped with 15 ml of chloroform/methanol/water (1/2/0.8 by volume) and washed with 10 ml of 0.1 N sodium bicarbonate followed by 10 ml of 0.1 N HCl. The lower chloroform phase was dried over anhydrous sodium sulfate and the chloroform was removed in vacuo.

The compound was recrystallized from chloroform/acetone at -20°C and further purified by preparative thin layer chromatography using silica gel G plates (250 micron thickness, Analtech, Newark, Delaware) developed with chloroform/methanol/water (65/35/6 by volume). In this system the product had an Rf of 0.62.

The product was further characterized by fast atom bombardment spectroscopy (FAB); two major molecular species were identified based on 16:0 m/z 658 and 18:0 m/z 686. Furthermore, a byproduct having two salicylate moieties at the sn-2 position of glycerol was identified by FAB having m/z 778 (corresponding to 16:0) and m/z 806 (corresponding to 18:0).

Alternatively, when aspirin anhydride (Fluka Chemical Co., Ronkonkoma, NY) is substituted for aspirin chloride and 4-dimethylaminopyridine (Aldrich Chemical Co., Milwaukee, Wisconsin) is used as the catalyst, a 3 to 4 fold higher yield was obtained.

EXAMPLE 2

Synthesis of 1-acyl,2-(2'-hydroxycarboxybenzoyl)-*sn*-glycero-3-phosphocholine

The symmetrical anhydride of salicylic acid was prepared by the method of Selinger and Lapidot. Briefly,

3 gms of salicylic acid, 0.5 gms of DCC (dicyclohexylcarbodiimide) in 20 ml of dry CCl_4 was stirred at room temperature for 5 hours. At the end of the reaction period, dicyclohexylurea was filtered off and the salicylic anhydride in the filtrate was isolated by evaporation and redissolved in 5ml of dry pyridine. A quantity of 0.3 gms of 4-dimethylaminopyridine and 0.12 gms of lysophosphatidylcholine was added to the dissolved salicylic anhydride and the reaction mixture was stirred overnight at room temperature under nitrogen. At the end of the reaction period 50 ml of diethylether was added and the precipitate removed by filtration. The filtrate was washed three times with 0.1N HCL, dried over anhydrous sodium sulphate, and evaporated under nitrogen. The isolated compound was redissolved in 30 ml of chloroform: methanol (1:1 V/V) and loaded on the silica column (1x18) with a bed depth of 6. The compound was eluted with 1.3L of chloroform: methanol (1:1 V/V) and then with 1.5L of chloroform: methanol:water (1:1:0.1, V/V). 15 ml fractions were collected. The pure compound was present in fraction numbers 86-136 and gave an Rf value of 0.41 on silica gel G plates developed with a chloroform/methanol/water (65:35:6 v/v) system.

EXAMPLE 3

Synthesis of 1-acyl,2-[2-(4-isobutylphenyl)propionyl]-sn-glycerol-3-phosphocholine

The symmetrical anhydride of 2-(4-isobutylphenyl) propionic acid (ibuprofen) was prepared as described in Example 2. 0.45 gm of the anhydride was dissolved in 5.0 ml of dry pyridine; 0.12 gm of 1-palmitoyl lysophosphatidylcholine and 400 mg of 4-dimethylaminopyridine in 20 ml of dry chloroform was added to the reaction mixture in a 100 ml round bottom flask.

The reaction mixture was sealed under nitrogen and allowed to stir overnight at room temperature. The reaction was stopped by the addition of 15 ml of chloroform/methanol/water (1/1/0.9 by volume) and the organic phase was removed and washed successively twice with 10 ml of 0.1 N HCl and twice with 0.1 sodium bicarbonate. The washed organic phase was separated, dried with anhydrous sodium sulfate and evaporated in vacuo. The product was purified by thin layer chromatography using 500 micron thickness silica gel G plates developed with chloroform/methanol/water (65/35/6 by volume). The Rf value of the pure compound was 0.27.

EXAMPLE 4

1,2-dioleoyl-sn-glycero-3-(2'-acetoxycarboxybenzoyl)-2-amidoethyl phosphate

A quantity of 300 mg of dioleoyl phosphatidylethanolamine (Avanti Polar Lipids, Pelham, Alabama); 73 mg of acetyl salicylic acid (Aldrich Chemical) and 83 mg of dicyclohexylcarbodiimide (DCC) (Aldrich Chemical) were added to a 200 ml round bottom flask. After the addition of 5 ml of dry pyridine and 5 ml of freshly distilled dry chloroform, the reaction mixture was stirred overnight at room temperature. The reaction was stopped by the addition of chloroform/methanol/water and the lipids were extracted into the organic phase as described above in Example 1. The organic phase was evaporated to a small volume in vacuum and chromatographed on silica gel G plates developed with chloroform/methanol/water:65/35/6 v/v, with an Rf value of 0.86. The product was purified by preparative TLC using 500 micron thick layers of silica gel G developed with the same system. The same analogue was also synthesized by using acetyl salicylic anhydride and 4-dimethylaminopyridine as described in Example 2.

EXAMPLE 5

1-acyl, 2-(2'-acetoxycarboxybenzoyl)-sn-glycero-3-(2'-acetoxycarboxybenzoyl)-2-amidoethyl phosphate

A quantity of 25 mg of lysophosphatidylthanolamine (Sigma Chemical, St. Louis, Missouri); 39 mg of acetyl salicylic anhydride (Fluka) and 14 mg of 4-dimethylaminopyridine was dissolved in 1 ml of pyridine and 10 ml of dry chloroform. The reaction mixture was stirred overnight at room temperature. The reaction mixture was processed as described above and the desired compound gave an Rf value of 0.64 when chromatographed on silica gel G plates (chloroform/methanol/water:65/35/6). Synthesis of this analogue was also accomplished by coupling aspirin to amino group by the dicyclohexylcarbodiimide method (DCC, Aldrich Chemical, Milwaukee, Wisconsin) as described in Example 4, followed by acylation of the 2-OH group with aspirin chloride.

Although the invention has been described in the context of particular embodiments, it is intended that the scope of coverage of the patent not be limited to those particular embodiments, but be determined by reference to the following claims.

APPENDIX:NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Generic Name	U.S.Patent	Brand Name	Company Name
sulindac	3,654,349/ 3,647,858	Clinoril	Merck
indomethacin	3,161,654	Indocin/ Interban	Merck/Sumimoto Chemical
ibuprofen	3,228,831	Brufen/ Motrin	Boots/Upjohn
ketoprofen		Orudis/ Profenid	Rhone-Poulenc
diflunisal	3,714,226	Dolobid	Merck
tolmetin	FR1,574,570	Tolectin	McNeil

Sodium 1-methyl-5-(4-methylbenzoyl)-1*H*-pyrrole-2-acetate dihydrate.

APPENDIX: NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Generic Name	U.S.Patent	Brand Name	Company Name
naproxen sodium	3,637,767	Naprosyn Anaprox	Syntex
diclofenac		Voltaren	Ciba-Geigy
oxaprozin		Alvo	Taisho
meclofenamate	3,313,848	Meclomen	Warner-Lambert
fenbufen		Cinopal	American Cyanamid
pirprofen		Rengasil	Ciba-Geigy
carprofen		Imadyl	Hoffman-La Roche
lobenzarit		Carfenil	Chugai-Upjohn
flurbiprofen		Ansaid	Upjohn
oxaprozin		Duzapro	American Home Products
nabumetone		Relifex	Beecham
Ioxoprofen		Loxonin	Sankyo
etodolac		Ultradol	American Home Products
loxoprofen		Loxonim	Sankyo
tenoxicam		Tilcoril	Hoffmann-La Roche
fenoprofen	3,600,437	Nalfon	Dista
oxindanac			Ciba-Geigy
nabumetone			Fujisawa
indobufen		Ibustrin	Erbamont
dibemoprofen			DaiNippon
nabumetone			Fusisawa
tiaprofenic acid		Surgam	Hoechst/Roussel
loxoprofen		Loxonin	Sankyo
flurbiprofen		Froben	Boots
pranoprofen		Nirlan	Yoshitomi

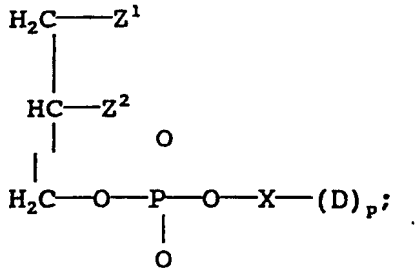
WHAT IS CLAIMED IS:

1. An anti-inflammatory agent comprising

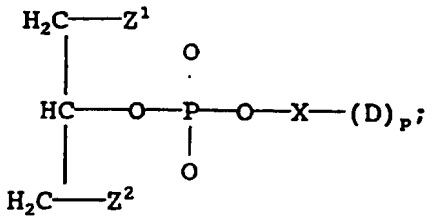
a salicylate or a nonsteroidal anti-inflammatory drug; and

a phospholipid species linked thereto.

2. A phospholipid derivative of a salicylate or a nonsteroidal anti-inflammatory drug having the formula:



; or



wherein D is a salicylate or a nonsteroidal anti-inflammatory drug;

Z is $(D)_n$, OH, or R,

wherein R is a C_2 to C_{24} aliphatic group in ester, ether, or vinyl ether linkage, having from 0 to 6 sites of unsaturation and the structure:

$CH_3-(CH_2)_a-(CH=CH-CH_2)_b-(CH_2)_c-Y$

and wherein the sum of a and c is from 1 to 23; and b is 0 to 6; and wherein Y is C(O)O-, C-O-, C=C-O-, C(O)S, C-S, or C=C-S;

except that Z^1 and Z^2 are not both OH;

n = 0 to 2; p = 0 to 2; p + n > 0; and

X is a phospholipid head group or is absent, providing that when X is absent, p = 0.

3. A compound according to Claim 1 or 2 wherein X is selected from the group consisting of choline, ethanolamine, glycerol, inositol, or serine.

4. The compound of Claim 1 or 2 wherein D is selected from the group consisting of 1-acetylsalicylic acid (aspirin); 2-((2,6-dichlorophenyl)amino)benzeneacetic acid, monosodium salt (diclofenac); (Z)-5-fluoro-2-methyl-1-((p-methylsulfinyl)phenyl)methylene)1-H-indene-3-acetic acid (sulindac); 2', 4'-difluoro-4-hydroxy-3-biphenylcarboxylic acid (diflunisal); 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1-H-indole-3-acetic acid (indomethacin); (±)-2-(p-isobutylphenyl)propionic acid (ibuprofen); N-(2), 6-dichloro-m-tolyl) anthranilic acid (meclophenomate); fenoprofen (Nalfon); 2-naphthaleneacetic acid, 6-methoxy-alpha-methyl-,(+) (naproxyn); 1-methyl-5-(4-methylbenzoyl)-1-H-pyrrole-2-acetate dihydrate (tolmetin); or derivatives thereof.

5. A compound according to Claim 1 or 2 which is 1-acyl, (2'-acetoxycarboxybenzoyl)phosphatidyl choline.

6. A compound according to Claim 1 or 2 which is 1-acyl, 2-(2'-hydroxycarboxybenzoyl)salicyloyl)-sn-glycerol-3-phosphocholine.

7. A compound according to Claim 1 or 2 which is 1-acyl, 2-(2-(4-isobutylphenyl)propionyl)-sn-glycerol-3-phosphocholine.

8. A compound according to Claim 1 or 2 which is 1,2-dioleoyl-sn-glycero-3-(2'-acetoxycarboxybenzoyl)2-amidoethyl phosphate.

9. A compound according to Claim 1 or 2 which is 1-acyl,2-(2'-acetoxycarboxybenzoyl)-sn-glycero-3-(2-acetoxycarboxybenzoyl)-2-amidoethyl phosphate.

10. A preparation for enteral use comprising a liposomal preparation of a therapeutic agent and any one of the compounds of Claim 1 through 9.

11. A liposome formed at least in part from any one of the compounds of Claims 1 through 10.

12. A liposomal formulation for enteral use, comprising a liposome formed at least in part from a phospholipid

prodrug having the structure set forth in Claim 2 in a pharmacologically acceptable vehicle.

13. A method of synthesizing an anti-inflammatory phospholipid prodrug having the structure set forth in Claim 2, comprising the step of reacting a non-steroidal anti-inflammatory drug, said drug having a functional linking group, with a phospholipid in the presence of a coupling reagent, whereby said drug is joined to said phospholipid to form a compound according to any one of Claims 1 through 10.

14. The method of Claim 13, wherein said linking group is a carboxylic acid.

15. The method of Claim 13, wherein said phospholipid is a diacylphospholipid, a 1-acyl, 2-lysophospholipid, a 1-lyso, 2-acylphospholipid, a 1-acyl, 3-lysophoSpholipid, 1-lyso, 3-acylphospholipid, or glycerol phosphate.

16. A method according to Claim 13 wherein said anti-inflammatory agent is in the form of a dimer.

17. The method of Claim 13 wherein said anti-inflammatory agent is acetylsalicylic acid.

18. The method of Claim 13 wherein said anti-inflammatory agent is 1-acyl,2-(2-(4-isobutylphenyl)propionyl)-sn-glycerol-3-phosphocholine.

19. A method of treating a patient having a chronic inflammatory disease, comprising the administration of an effective inflammation-reducing dose of a phospholipid prodrug having the structure set forth in Claim 2, said administration being at regular intervals and for a period of time sufficient to alleviate the symptoms of said disease.

20. The method of Claim 19, wherein said disease is rheumatoid arthritis or osteoarthritis.

21. A method of enhancing the oral administration of therapeutic agents by co-administration of a phospholipid prodrug having the structure set forth in Claim 2.

22. The method of Claim 19, 20, or 21 wherein said phospholipid prodrug is in liposomal form.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US91/02447

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/22
U.S. CL. 424/450

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	260/403; 424/450, 464, 489; 514/78; 558/177, 179, 181

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [6]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	J. Pharm. Pharmac. Volume 30, 1978, KUMAR, "Gastric ulceration and the concentration of salicylate in plasma in rats after administration of ^{14}C-labelled aspirin and its synthetic triglyceride, 1,3-dipalmitoyl 2(2'acetoxy-[^{14}C] carboxyl-benzoyl) glycerol,", See pages 754-758.	1-12, 19-22
Y	US, A, 3,644,424 (SHERLOCK) 22 FEBRUARY 1972; See the Abstract and column 5, lines 17-28.	1-12, 19-22
Y	US, A, 3,988,446 (PARIS) 26 OCTOBER 1976; See the Abstract, column 1, line 29 through column 3, line 29 and the Examples.	1-12, 19-22
A	US, A, 4,129,650 (BETZING) 12 DECEMBER 1978; See the entire document.	
A	US, A, 3,746,728 (GORDON) 17 JULY 1973; See the entire document.	

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [1]	Date of Mailing of this International Search Report [2]
28 June 1991	20 AUG 1991
International Searching Authority [3]	**Signature of Authorized Officer [20]**
ISA/US	Thurman K. Page

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1 ☐ Claim numbers . because they relate to subject matter[2] not required to be searched by this Authority, namely:

2. ☒ Claim numbers 13-18 . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out[3], specifically:

These "method of synthesis claims" are recited in vague terms without giving details of the synthesis steps or the functional groups reacting. No meaningful search can be made for these claims as recited.

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[4]

This International Searching Authority found multiple inventions in this international application as follows:

This application contains the following patentably distinct species
1) aspirin 2) diclofenac 3) sulindac 4) diflunisal 5) indomethacin
6) meclophenomate 7) nalfon 8) naproxyn 9) tolmetin 10) ibuprofen

(Telephone practice)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

aspirin in claim 4.

4 ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

This application contains the following patentably distinct species:

1) aspirin
2) diclofenac
3) sulindac
4) diflunisal
5) indomethacin
6) meclophenomate
7) nalfon
8) naproxyn
9) tolmetin
10) ibuprofen

These are deemed to be patentably distinct in view of their structural dissimilarities and the use in the treatment of diverse diseases.